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## **FASTKD2 is associated with memory and hippocampal structure in older adults**

Vijay K Ramanan, Ph.D.<sup>1,2,3</sup>, Kwangsik Nho, Ph.D.<sup>1</sup>, Li Shen, Ph.D.<sup>1,4</sup>, Shannon L. Risacher, Ph.D.<sup>1</sup>, Sungeun Kim, Ph.D.<sup>1</sup>, Brenna C. McDonald, Psy.D.<sup>1,5,6</sup>, Martin R. Farlow, M.D.<sup>5,6</sup>, Tatiana M. Foroud, Ph.D.<sup>1,2,4,6</sup>, Sujuan Gao, Ph.D.<sup>6,7</sup>, Hilka Soininen, M.D., Ph.D.<sup>8,9</sup>, Iwona Kłoszewska, M.D., Ph.D.<sup>8,10</sup>, Patrizia Mecocci, M.D., Ph.D.<sup>8,11</sup>, Magda Tsolaki, M.D., Ph.D.<sup>8,12</sup>, Bruno Vellas, M.D., Ph.D.<sup>8,13</sup>, Simon Lovestone, Ph.D., MRCPsych<sup>8,14</sup>, Paul S. Aisen, M.D.<sup>15</sup>, Ronald C. Petersen, M.D.<sup>16</sup>, Clifford R. Jack Jr., M.D.<sup>17</sup>, Leslie M. Shaw, Ph.D.<sup>18,19</sup>, John Q. Trojanowski, Ph.D.<sup>18,19</sup>, Michael W. Weiner, M.D.<sup>20,21</sup>, Robert C. Green, M.D., M.P.H.<sup>22</sup>, Arthur W. Toga, Ph.D.<sup>23</sup>, Philip L. De Jager, M.D., Ph.D.<sup>24,25,26</sup>, Lei Yu, Ph.D.<sup>27</sup>, David A. Bennett, M.D.<sup>27</sup>, Andrew J. Saykin, Psy.D.<sup>1,2,4,6,\*</sup>, and the Alzheimer's Disease Neuroimaging Initiative (ADNI)<sup>Ω</sup>

<sup>1</sup>Center for Neuroimaging, Department of Radiology and Imaging Sciences, Indiana University School of Medicine, Indianapolis, IN, USA <sup>2</sup>Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA <sup>3</sup>Medical Scientist Training Program, Indiana University School of Medicine, Indianapolis, IN, USA <sup>4</sup>Center for Computational Biology and Bioinformatics, Indiana University School of Medicine, Indianapolis, IN, USA <sup>5</sup>Department of Neurology, Indiana University School of Medicine, Indianapolis, IN, USA <sup>6</sup>Indiana Alzheimer's Disease Center, Indiana University School of Medicine, Indianapolis, IN, USA <sup>7</sup>Department of Biostatistics, Indiana University School of Medicine, Indianapolis, IN, USA <sup>8</sup>On behalf of the AddNeuroMed Consortium <sup>9</sup>Department of Neurology, University of Eastern Finland and Kuopio University Hospital, Kuopio, Finland <sup>10</sup>Medical University of Lodz, Lodz, Poland <sup>11</sup>Institute of Gerontology and Geriatrics, University of Perugia, Perugia, Italy <sup>12</sup>3rd Department of Neurology, Aristotle University, Thessaloniki, Greece <sup>13</sup>INSERM U 558, University of Toulouse, Toulouse,

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\*Correspondence to: Dr. Andrew J. Saykin, IU Health Neuroscience Center, Suite 4100 Indiana University School of Medicine 355 West 16<sup>th</sup> Street, Indianapolis, IN 46202, USA [asaykin@iupui.edu](mailto:asaykin@iupui.edu), Phone (317)963-7501, Fax (317)963-7547.

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### AUTHOR CONTRIBUTIONS

All authors contributed substantively to this report. VKR, LS, and AJS were involved in study conception and design. HS, IK, PM, MT, BV, and SL were involved in coordination and data collection and processing for AddNeuroMed. PSA, RCP, CRJ, LMS, JQT, MWW, RCG, AWT, and AJS were involved in coordination and data collection and processing for ADNI. BCM, MRF, TMF, SG, and AJS were involved in coordination and data collection and processing for IMAS. PLDJ, LY, and DAB were involved in coordination and data collection and processing for ROS and MAP. VKR, KN, LS, SLR, SK, TMF, SG, and AJS were involved in data organization and planning and execution of statistical analyses. VKR and AJS drafted the report and prepared all figures and tables. All authors were involved in reviewing and editing of the report.

### POTENTIAL CONFLICTS OF INTEREST

The authors declare no potential conflicts of interest.

France <sup>14</sup>University of Oxford, Department of Psychiatry, Oxford, UK <sup>15</sup>Department of Neuroscience, University of California-San Diego, San Diego, CA, USA <sup>16</sup>Department of Neurology, Mayo Clinic Minnesota, Rochester, MN, USA <sup>17</sup>Department of Radiology, Mayo Clinic Minnesota, Rochester, MN, USA <sup>18</sup>Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA, USA <sup>19</sup>Institute on Aging, University of Pennsylvania School of Medicine, Philadelphia, PA, USA <sup>20</sup>Departments of Radiology, Medicine, and Psychiatry, University of California-San Francisco, San Francisco, CA, USA <sup>21</sup>Department of Veterans Affairs Medical Center, San Francisco, CA, USA <sup>22</sup>Division of Genetics, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA <sup>23</sup>Laboratory of Neuro Imaging, Keck School of Medicine, University of Southern California, Los Angeles, CA USA <sup>24</sup>Program in Translational NeuroPsychiatric Genomics, Institute for the Neurosciences, Departments of Neurology and Psychiatry, Brigham and Women's Hospital, Boston, MA, USA <sup>25</sup>Harvard Medical School, Boston, MA, USA <sup>26</sup>Program in Medical and Population Genetics, Broad Institute, Cambridge, MA, USA <sup>27</sup>Rush Alzheimer's Disease Center, Rush University Medical Center, Chicago, IL, USA

## Abstract

Memory impairment is the cardinal early feature of Alzheimer's disease (AD), a highly prevalent disorder whose causes remain only partially understood. To identify novel genetic predictors, we used an integrative genomics approach to perform the largest study to date of human memory ( $n=14,781$ ). Using a genome-wide screen, we discovered a novel association of a polymorphism in the pro-apoptotic gene *FASTKD2* (fas-activated serine/threonine kinase domains 2; rs7594645-G) with better memory performance and replicated this finding in independent samples. Consistent with a neuroprotective effect, rs7594645-G carriers exhibited increased hippocampal volume and gray matter density and decreased cerebrospinal fluid levels of apoptotic mediators. The *MTOR* (mechanistic target of rapamycin) gene and pathways related to endocytosis, cholinergic neurotransmission, EGFR signaling, and immune regulation, among others, also displayed association with memory. These findings nominate *FASTKD2* as a target for modulating neurodegeneration and suggest potential mechanisms for therapies to combat memory loss in normal cognitive aging and dementia.

## Keywords

genome-wide association study (GWAS); Alzheimer's disease (AD); dementia; cognitive aging; memory; magnetic resonance imaging (MRI); apoptosis; neurodegeneration

## INTRODUCTION

Human memory is a complex, dynamic trait with significant roles in development, aging, and disease. Impairment in episodic memory – involving the encoding and conscious recollection of experiences – is an early hallmark of AD, the most common cause of dementia.<sup>1</sup> Declines in episodic and other memory domains are also found in normal cognitive aging and many age-related disorders, including Parkinson's disease, diabetes, and

cancer.<sup>2</sup> With the rising incidence and burdens of dementia, a better understanding of its causes is crucial for risk stratification and the development of memory-sparing lifestyle and drug therapies.<sup>3, 4</sup>

At present, the molecular mechanisms underlying memory performance are not fully understood. Epidemiological studies have linked many factors to memory, including the presence of vascular and metabolic disease, mental and physical activity, and educational and occupational attainment.<sup>5, 6</sup> Episodic memory is estimated to have substantial heritability (30-60%) based on twin studies and is thought to be influenced by common and rare genetic variation in multiple pathways.<sup>6</sup> Although genetic studies have implicated numerous single nucleotide polymorphisms (SNPs) in episodic memory performance,<sup>7</sup> significant heritability remains unexplained.

Recently, there has been great interest in harnessing the power of genome-wide association studies (GWAS) to gain additional mechanistic insight into complex neurological traits and diseases.<sup>8</sup> Extant approaches for maximizing the translational impact of GWAS findings include using large samples with adequate statistical power for discovery,<sup>6</sup> performing complementary analyses to detect broader trends of association within genes and pathways,<sup>9</sup> and assessing for convergence of genetic associations across phenotypic modalities to understand their biological context.<sup>10, 11</sup> Here, we integrated GWAS with molecular and neuroimaging data to identify and characterize novel predictors of episodic memory function in older adults.

## MATERIALS AND METHODS

### Study participants

Launched in 1992, the Health and Retirement Study (HRS) is a nationally representative longitudinal study of more than 26,000 Americans over 50 years old.<sup>12</sup> Biennial interviews were used to collect detailed information on the health, social, and economic status of participants.

The AddNeuroMed Consortium,<sup>13</sup> Alzheimer's Disease Neuroimaging Initiative (ADNI),<sup>14</sup> Indiana Memory and Aging Study (IMAS),<sup>15</sup> Rush Memory and Aging Project (MAP),<sup>16</sup> and Religious Orders Study (ROS)<sup>17</sup> include older adults representing clinical stages along the continuum from normal aging to AD who received longitudinal clinical evaluations.

All participants provided written informed consent and study protocols were approved by each site's institutional review board.

### Clinical and cognitive assessment data

We obtained the RAND Corporation's public release HRS data files (version L) which integrated information across study waves.<sup>18</sup> For discovery, we analyzed HRS wave 3 since it contained the largest cross-sectional sample with complete data for cognitive functioning variables. To maximize statistical power for discovery, we analyzed the full available sample including multiple ethnicities and included principal component eigenvectors to control for population stratification.<sup>19</sup> Episodic memory performance was assessed through

immediate free recall of a 10-word list.<sup>20</sup> Age, gender, and years of education were used as covariates for GWAS.

For replication, we used independent samples from HRS wave 7, AddNeuroMed, ADNI, IMAS, MAP, and ROS. With the exception of HRS, word list immediate recall tasks included multiple trials and therefore the sum of those trials at the baseline visit was used as the outcome. Immediate recall tasks used in these cohorts included the Rey Auditory Verbal Learning Test (RAVLT) in ADNI, the California Verbal Learning Test (CVLT) in IMAS, and the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) word list test in AddNeuroMed, MAP, and ROS. Age, gender, and education were included as covariates in all replication analyses. Diagnosis (as a categorical variable) was also included as a covariate for analyses in AddNeuroMed, ADNI, IMAS, MAP, and ROS to account for the clinical stages represented among participants.

### Structural magnetic resonance imaging (MRI) data

Baseline visit T1-weighted cranial MRI scans were analyzed for 315 healthy control participants from ADNI who were not included in the memory analyses. Scan protocols, quality control, image processing, and extraction of volume and gray matter density for brain regions of interest were performed as described previously.<sup>21</sup>

### Cerebrospinal fluid (CSF) proteomic data

Baseline visit CSF samples were analyzed for 82 healthy control participants from ADNI who were not included in the memory analyses. Sample acquisition and quality control procedures were performed as described previously.<sup>22</sup> Levels for 159 proteins were measured by multiplex immunoassay on the Myriad Genetics (Austin, TX) Rules Based Medicine Human Discovery MAP panel. Stringent quality control for protein level measurements was performed as described previously.<sup>23</sup> Additional technical details are available in the ADNI Biomarker Consortium CSF Proteomics Data Primer (<http://adni.loni.usc.edu/wp-content/uploads/2012/01/2011Dec28-Biomarkers-Consortium-Data-Primer-FINAL1.pdf>). Based on literature search, we performed a pathway-guided analysis of CSF levels of four proteins (adiponectin, fas ligand, TNFR2, and TRAIL-R3) involved in fas-mediated apoptosis. Protein levels were log<sub>10</sub>-transformed to ensure a normal distribution of each phenotype.

### Genotyping and imputation

Genomic DNA for 12,507 participants was obtained during HRS waves 8-9 via buccal swab or saliva sample. Genotyping was performed at the Center for Inherited Disease Research (CIDR; Johns Hopkins University) using the Illumina (San Diego, CA) HumanOmni2.5-4v1 array. Genotype data underwent stringent quality control including identity checks, sample exclusion for call rate < 98%, and SNP exclusion for call rate < 98%, Hardy-Weinberg  $p < 10^{-4}$ , minor allele frequency (MAF) < 1%, or failure of other technical and quality filters.<sup>24</sup> Identity by descent analysis in PLINK<sup>25</sup> (<http://pngu.mgh.harvard.edu/~purcell/plink/>) revealed 80 pairs and 4 trios with significant relatedness; therefore one participant from each unit was randomly selected for inclusion. SNPRelate<sup>26</sup> was used to identify population group outliers and generate principal components to control for population substructure. As described

previously, on scree plot the fraction of variance explained was minimal and level after the first two principal components.<sup>24</sup> The final dataset included 1,681,327 SNPs for 12,419 participants.

We used Minimac<sup>27</sup> and the 1000 Genomes Project to impute the SNPs characterizing the *APOE*  $\epsilon 4$  allele (rs429358, rs7412) in HRS and the specific SNPs required for replication studies in AddNeuroMed, ADNI, IMAS, MAP, and ROS. Imputation and quality control were performed as described previously.<sup>28, 29</sup> Due to the control for population substructure required for imputation quality, imputation was restricted to participants with non-Hispanic Caucasian ancestry as determined by multidimensional clustering in PLINK.<sup>28</sup>

## Statistical analysis

Basic statistical analyses were performed using IBM SPSS Statistics for Windows, Version 22.0 (Armonk, NY). Genetic associations were tested using linear regression under an additive genetic model in PLINK. In the discovery sample GWAS, the first three principal components were included as covariates consistent with prior studies of AD endophenotypes,<sup>30, 31</sup> and a conservative significance threshold ( $p < 5 \times 10^{-8}$ ) was employed based on a Bonferroni correction of one million independent tests.<sup>32</sup> For analysis of the HRS replication sample (wave 7), the first three principal components were included as covariates as in the discovery GWAS. For all other replication analyses, the potential effects of population stratification were minimized through restricting analyses to participants with non-Hispanic Caucasian ancestry as determined by multidimensional clustering in PLINK.<sup>28</sup> Manhattan and Q-Q plots were generated with Haploview<sup>33</sup> and regional association and linkage disequilibrium (LD) plots were generated with LocusZoom<sup>34</sup> and SNAP.<sup>35</sup>

Complementary approaches were used to validate and extend the GWAS findings. We used HYST<sup>36</sup> to calculate a summary  $p$ -value for each gene accounting for its size, LD structure, and constituent GWAS SNP associations. We then utilized these summary  $p$ -values and GSA-SNP<sup>37</sup> to identify pathways enriched with significant association to memory. Pathway definitions, consisting of canonical representations of biological processes compiled by expert reviewers, were downloaded from the Molecular Signatures Database (<http://www.broadinstitute.org/gsea/msigdb/index.jsp>; canonical pathways set, version 4.0) and the false discovery rate (FDR) was used to correct for pathway-level multiple comparisons.

Genome-wide significant associations were further investigated in independent samples through inverse-variance weighted meta-analysis using METAL<sup>38</sup> and through association testing with MRI and CSF biomarkers using multivariate analyses of covariance with Wilks' lambda as the test statistic. For meta-analysis, effect sizes were denoted by standardized  $\beta$  coefficients from linear regression. Cohen's  $d$  statistics were also calculated to facilitate comparison of effect sizes across phenotypes.

## RESULTS

### Study participants

This study involved a total of 14,781 participants from six independent cohorts (Table 1). For discovery, we analyzed data for 6,705 participants from HRS wave 3. In the HRS,

immediate recall was approximately normally distributed (Supplementary Figure 1) and was highly-correlated with delayed recall ( $r=0.74$ ). Using linear regression, younger age ( $p=3.73\times 10^{-56}$ ), female gender ( $p=2.70\times 10^{-41}$ ), and more years of education ( $p=4.74\times 10^{-170}$ ) were associated with better episodic memory performance and collectively explained 16.4% of the variance in immediate recall (omnibus  $F(3, 6701)=437.88$ ,  $p=8.77\times 10^{-260}$ ).

### GWAS of episodic memory

To identify novel genetic predictors of episodic memory functioning, we performed a GWAS ( $n=6,705$ ) controlling for age, gender, education, and the first three principal components to address population substructure. The genomic inflation factor ( $\lambda$ ) was 1.007, indicating no substantial inflation of association (Supplementary Figure 2).

A genome-wide significant association with immediate recall was identified on chromosome 2 for rs7594645 ( $p=3.11\times 10^{-9}$ ; Figure 1). This SNP resides in an intron of *FASTKD2* that is overlapped by *MIR3130-1* and *MIR3130-2* (Figure 2a). The rs7594645-G allele exhibited a modest additive effect associated with better episodic memory performance and explained an additional 0.5% of the phenotypic variance (Figure 2b).

Suggestive associations with immediate recall ( $p<5\times 10^{-6}$ ) were also identified (Figure 1; Supplementary Table 1). These included SNPs near *FASTKD2* within *DYTN* (dystrotelin) as well as SNPs on additional chromosomes within *LRRC38* (leucine rich repeat containing 38), *SRC* (v-Src tyrosine kinase), and *APOL2* (apolipoprotein L2). We also observed nominal associations with immediate recall ( $p<0.05$ ) for 14 of 25 memory- and AD-related candidate genes identified through literature review (Supplementary Table 2). These genes included *BCHE* (butyrylcholinesterase), *BDNF* (brain-derived neurotrophic factor), *CR1* (complement receptor 1), and *TREM2* (triggering receptor expressed on myeloid cells 2), among others. Significant association after Bonferroni correction for 25 genes ( $p<0.002$ ) was observed for three of these genes, *CAMTA1* (calmodulin binding transcription activator 1), *DISC1* (disrupted in schizophrenia 1), and *WWC1* (WW and C2 domain containing 1; also known as KIBRA).

Due to its well-known association with AD,<sup>39</sup> we further investigated the effect of the *APOE* (apolipoprotein E)  $\epsilon 4$  allele in the GWAS sample. Since the SNPs characterizing *APOE*  $\epsilon 4$  (rs429358, rs7412) failed initial genotyping quality control, to perform additional analyses we imputed these SNPs in non-Hispanic Caucasian participants ( $n=5,288$ ). We did not observe an association of *APOE*  $\epsilon 4$  with immediate ( $p=0.904$ ) or delayed ( $p=0.466$ ) recall in this sample. However, *APOE*  $\epsilon 4$  was associated with increased odds of self-reported diagnosis of AD by a doctor ( $p=5.96\times 10^{-13}$ , OR 5.59, 95% CI 3.50-8.91) among participants re-evaluated 14 years later ( $n=4,491$ ).

### Gene- and pathway-based analyses of episodic memory

To extend the GWAS findings, we performed complementary analyses at the gene and pathway levels. Using HYST to calculate summary gene-based  $p$ -values, we identified additional genome-wide significant associations with episodic memory based on a



Bonferroni correction for 23,672 genes ( $p < 2.11 \times 10^{-6}$ ; Supplementary Table 3). Top genome-wide significant associations included the genes *LARS2* (leucyltRNA synthetase 2, mitochondrial;  $p = 5.56 \times 10^{-11}$ ) and *MTOR* ( $p = 2.50 \times 10^{-9}$ ), which along with *FASTKD2*<sup>40</sup> encode proteins with critical roles in mitochondrial function.<sup>41, 42</sup>

Numerous pathways exhibited enrichment of association (FDR-corrected  $p < 0.05$ ) with episodic memory performance (Table 2). These included multiple mechanisms related to immune regulation and cell adhesion as well as pathways related to cholinergic neurotransmission, endocytosis, and epidermal growth factor receptor (EGFR; ErbB1) signaling, among others.

### Replication of *FASTKD2* association with episodic memory

For replication of our major SNP-based genome-wide significant finding, we analyzed independent samples from the HRS, AddNeuroMed, ADNI, IMAS, MAP, and ROS cohorts. Due to heterogeneity of memory instruments and clinical populations, we initially analyzed within cohorts and then performed a replication meta-analysis involving 7,761 participants which validated the association of rs7594645-G with higher immediate recall ( $p = 0.013$ ; Supplementary Table 4 and Supplementary Figure 3). In all replication cohorts except ROS, rs7594645-G exhibited a positive direction of effect on episodic memory performance consistent with the discovery sample. The heterogeneity statistic ( $I^2$ )  $p$ -value was 0.71, indicating no significant heterogeneity across the replication studies.<sup>43</sup> Overall, a summary meta-analysis of rs7594645-G across the discovery and replication samples combined displayed genome-wide significant association ( $p = 3.73 \times 10^{-9}$ , total  $n = 14,466$ ).

### Association of rs7594645 with *FASTKD2* mRNA expression

We assessed the functional impact of rs7594645 on *FASTKD2* mRNA expression using two expression quantitative locus (eQTL) databases. Using Genevar,<sup>44</sup> we analyzed published eQTL data for 856 healthy female twins from the MuTHER (Multiple Tissue Human Expression Resource) project.<sup>45</sup> Although rs7594645 was not available in this dataset, we analyzed rs10490541 as a proxy SNP since its minor allele (T) is in complete LD ( $D' = 1$ ,  $r^2 = 1$ ) with rs7594645-G in the CEU reference population from the 1000 Genomes Project. We observed that rs10490541-T was associated with decreased *FASTKD2* mRNA expression in skin cells ( $p = 0.035$ ), controlling for participant age, experimental batch, and sample processing.<sup>45</sup> Using GeneNetwork,<sup>46</sup> we next analyzed published brain eQTL data for 187 healthy older adults.<sup>47, 48</sup> In this analysis, we identified a SNP (rs4578841) in strong LD with rs7594645 ( $D' = 1$ ,  $r^2 = 0.13$ ) that displayed significant association to neocortical *FASTKD2* expression ( $p = 9.16 \times 10^{-4}$ ).

### Association of rs7594645 with cerebrospinal fluid (CSF) levels of pro-apoptotic factors

*FASTKD2* encodes a pro-apoptotic protein that is predominantly localized to mitochondria.<sup>40, 49</sup> Given the association of rs7594645-G with higher memory performance and lower *FASTKD2* mRNA expression, we hypothesized that rs7594645-G would also be associated with decreased activation of apoptosis in the central nervous system. To test this hypothesis, we analyzed CSF levels of four proteins involved in fas-mediated apoptosis in 82 healthy control participants from ADNI (Supplementary Figure 4). Controlling for age

and gender, rs7594645-G carriers displayed decreased CSF levels of adiponectin ( $p=5.85\times 10^{-5}$ ) and fas ligand ( $p=0.012$ ) and trended towards decreased levels of TNFR2 (tumor necrosis factor receptor 2;  $p=0.057$ ) and TRAIL-R3 (tumor necrosis factor-related apoptosis inducing ligand receptor 3;  $p=0.083$ ), with a significant multivariate effect of genotype ( $p=1.07\times 10^{-4}$ ).

### Association of *FASTKD2* with hippocampal structure

Given the association of rs7594645-G with higher memory performance and the evidence of a functional molecular effect of this SNP, we hypothesized that *FASTKD2* would also be associated with structural changes in episodic memory-related brain regions. To test this hypothesis, we assessed hippocampal volume and gray matter density in 315 healthy control participants from ADNI who were genotyped and received high resolution T1-weighted structural MRI (Figure 3). Controlling for age, gender, education, and intracranial volume, rs7594645-G was associated with hippocampal structure (multivariate  $p=0.036$ ), including higher volume ( $p=0.034$ ) and gray matter density ( $p=0.019$ ).

## DISCUSSION

Using GWAS in a large, population-based sample of older adults, this study discovered a genome-wide significant association of *FASTKD2* rs7594645-G with better episodic memory performance. This novel association was replicated in independent samples and was further validated by converging evidence from structural MRI and gene and protein expression studies consistent with a neuroprotective effect in the brain.

*FASTKD2* encodes a pro-apoptotic protein<sup>40, 49</sup> and has not been previously associated with cognition. Signaling through its upstream activator and death receptor fas/CD95 is known to mediate neuronal responses to traumatic brain injury<sup>50</sup> and may have roles in neurodegeneration related to amyloid- $\beta$ ,<sup>51</sup> methamphetamines,<sup>52</sup> and frontotemporal lobar degeneration.<sup>53</sup> Rare *FASTKD2* mutations have also been linked to infantile encephalopathy associated with electron transport chain complex IV deficiency, suggesting a possible secondary role in cellular energetics.<sup>40</sup>

rs7594645 is an intronic variant in *FASTKD2* that exhibited an effect size comparable to most SNP associations for complex behavioral traits.<sup>54</sup> There is substantial precedent for non-coding intronic or intergenic variants to exert functional effects through regulation of chromatin structure, transcription factor binding, and splicing component recognition, among other mechanisms.<sup>55-57</sup> In addition, GWAS-implicated SNPs with modest effect sizes have highlighted druggable targets for type 2 diabetes, cancer, and other diseases, demonstrating the potential biological importance of replicable and convergent associations.<sup>57, 58</sup>

rs7594645 is located in a gene-dense region with extensive LD structure (Supplementary Figure 5). In particular, rs7594645 is overlapped by two genes encoding microRNA molecules. MicroRNAs are small, non-coding RNAs that base pair with complementary sequences in protein-coding mRNAs to direct their degradation or translational repression.<sup>59</sup> The location of rs7594645 suggests that allelic variation in this region may regulate



*FASTKD2* expression through altering mRNA-microRNA interactions. Based on the UCSC Genome Browser, rs7594645 is also 3 base pairs downstream from a binding sequence for CTCF (CCCTC-binding factor), a highly conserved transcription factor required for neuronal development and synapse formation.<sup>60</sup> Genetic variation at rs7594645 or a nearby tagging SNP may alter CTCF-mediated transcriptional regulation of *FASTKD2* to impact neuronal viability. Molecular characterization in brain tissue, cell cultures, and animal models will be important to assess and clarify these hypothesized mechanisms and the broader functional architecture around *FASTKD2* and its neighboring genes *DYTN*, *MDH1B*, and *MIR3130-1* and -2.

Based on the Human Brain Transcriptome, a database of genome-wide transcriptome data from postmortem human brains, *FASTKD2* is highly-expressed in the hippocampus throughout adulthood.<sup>61</sup> Behavioral, imaging, and pathological studies have revealed the hippocampus to be crucial for episodic memory storage and retrieval.<sup>62</sup> We identified a protective effect of rs7594645-G on hippocampal volume and gray matter density in older adults. Consistent with an anti-apoptotic, neuroprotective effect, we also identified an association of rs7594645-G with decreased *FASTKD2* mRNA expression and decreased CSF levels of proteins involved in apoptosis. These convergent findings at the molecular and neural systems levels further support the observed effect of rs7594645-G on memory function and suggest that modulation of *FASTKD2* and its functional pathways may be candidates for nootropic and/or neuroprotective drug development.

The overall allelic frequency of rs7594645-G in the discovery sample was 0.072. Among non-Hispanic Caucasian participants, which comprised 79% of this sample, the frequency of rs7594645-G was 0.064. Although rs7594645-G was relatively more common in African-American participants (MAF=0.139), this difference did not appear to exhibit a confounding effect on the association of rs7594645 with episodic memory performance (Supplementary Table 5). In the replication samples, rs7594645-G exhibited an overall frequency of 0.068 and displayed a positive effect on episodic memory performance in all cohorts except ROS. Greater levels of education, among other potential differences, may have modified the effect of rs7594645-G on episodic memory performance in the ROS sample.

Nominal associations for several memory- and AD-related candidate genes were observed in the discovery sample, including *WWC1*<sup>63</sup>, *DISC1*,<sup>64</sup> and the neurotransmission- or neurotrophic-related genes *BCHE*,<sup>28</sup> *BDNF*,<sup>65</sup> and *CAMTA1*.<sup>66</sup> Although in the discovery sample the *APOE* ε4 allele was associated with increased odds of self-reported diagnosis of AD by a doctor, no SNPs in *APOE* were associated with immediate recall. The *APOE* ε4 allele is the strongest known genetic risk factor for sporadic AD,<sup>39</sup> but its relationship to non-pathological cognitive aging is less clear.<sup>67, 68</sup> Alternative study designs might have detected effects of *APOE* on cognition, including cross-sectional analyses of older samples or other cognitive domains as well as longitudinal analyses to identify relative declines within individuals over time.<sup>67, 69</sup>

Gene- and pathway-based analyses provide additional vehicles for characterizing complex genetic architectures, since variants of modest individual effect can collectively influence susceptibility through action within shared mechanisms.<sup>9, 70, 71</sup> Through a gene-based

approach accounting for effects of multiple SNPs, we identified genome-wide significant associations of *LARS2* and *MTOR* with episodic memory that were otherwise concealed through standard SNP-based analysis. Mutations in *LARS2* have been implicated in Perrault Syndrome, a rare disease characterized by hearing loss and premature ovarian failure and proposed to be mediated by mitochondrial and apoptotic dysfunction.<sup>42</sup> The *MTOR* gene represents a major drug target for AD and other neurodegenerative disorders due to well-studied roles in amyloid and tau pathology.<sup>72, 73</sup> We also observed enrichment of association with episodic memory within the EGFR pathway, which has been proposed as a target for treating amyloid-induced memory loss,<sup>74</sup> as well as other mechanisms related to cholinergic neurotransmission, endocytosis, and the immune response.<sup>75, 76</sup>

Although this represents the largest known GWAS of memory performance in older adults, with a bigger sample the suggestive loci we highlighted might have reached genome-wide significance. These included *DYTN* which encodes a member of a protein family associated with schizophrenia and cognitive aging<sup>7</sup> and *APOL2* which functions with *APOE* in lipid pathways.<sup>77</sup> In addition, although the confirmatory and novel findings from this study validate its analytical design, alternative assessments of memory performance and clinical status might have yielded more sensitive phenotypic measures for GWAS.

Future extensions of this work using next-generation sequencing and related methods may reveal other associations with episodic memory, as novel SNPs, rare, structural, and epigenetic variants, and dynamic molecular changes in the transcriptome may all play a role in memory.<sup>78</sup> Studies of longitudinal change in episodic memory functioning may also elucidate additional mechanistic influences. Further, although it was beyond the scope of this study, characterizing gene-environment interactions will likely guide the optimized application of drug and lifestyle modifiers to preserve or enhance episodic memory.

In conclusion, this study discovered and validated a novel association of *FASTKD2* with episodic memory function and hippocampal structure. Overall, these new findings identify potential targets to help improve risk stratification and therapeutic development in normal cognitive aging and dementia.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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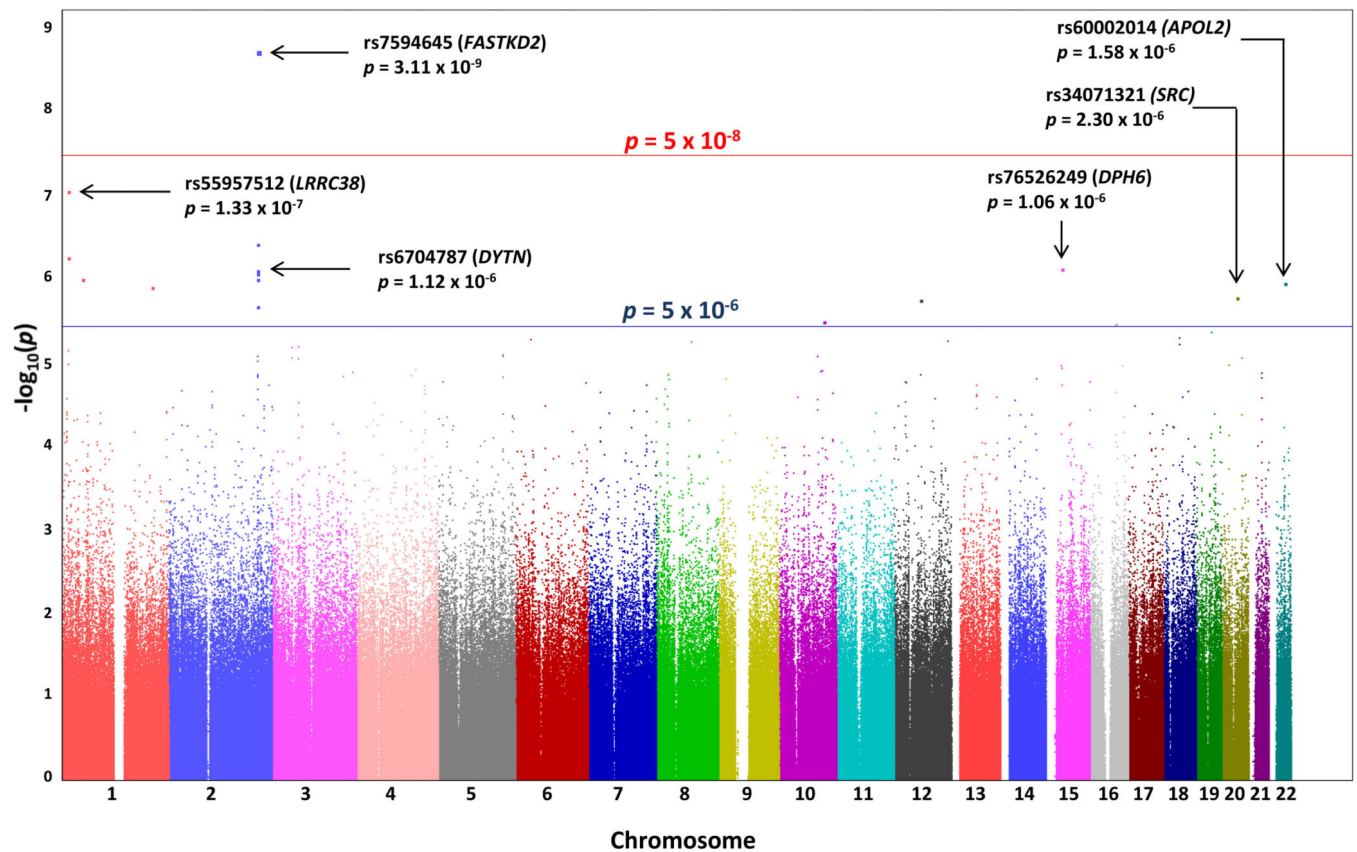
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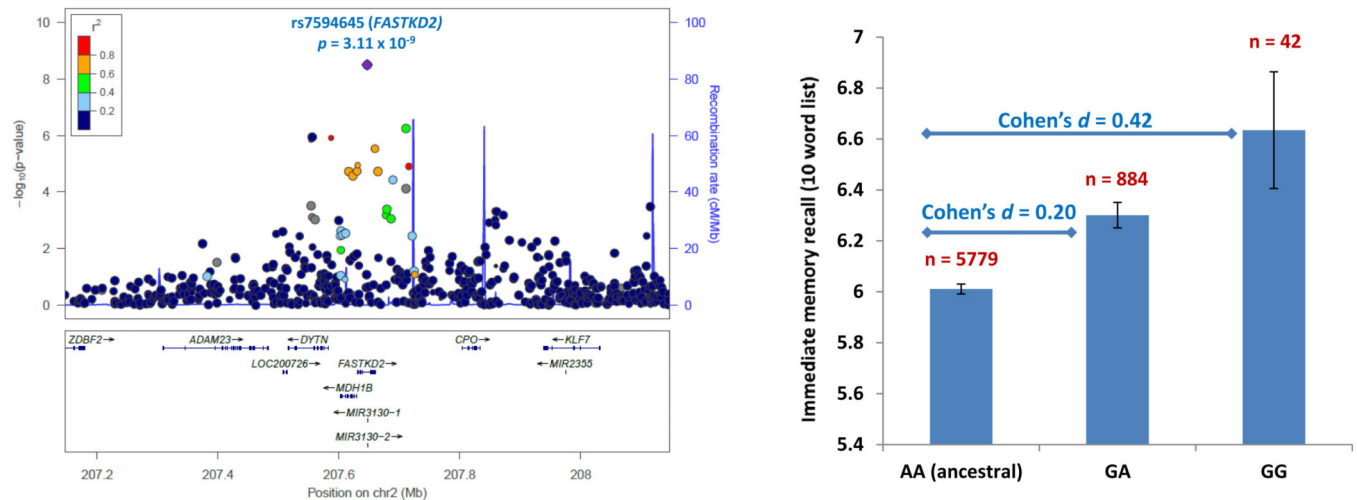


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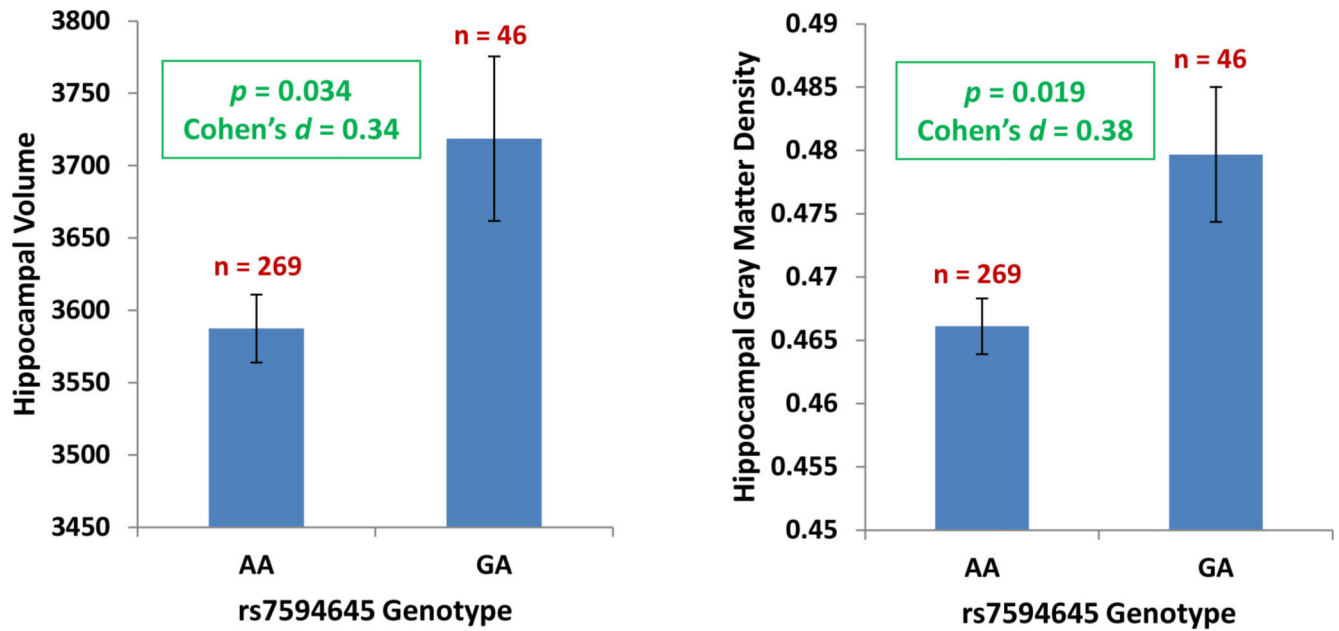
**Figure 1. Manhattan plot for the HRS discovery GWAS of immediate recall**

Observed  $-\log_{10}(p)$ -values (y-axis) are displayed for all tested SNPs on each chromosome (x-axis). A genome-wide significant association ( $p < 5 \times 10^{-8}$ ) to immediate recall was identified on chromosome 2 within *FASTKD2*. Suggestive associations ( $p < 5 \times 10^{-6}$ ) were identified on additional chromosomes. Selected top associations are labeled on the plot.



**Figure 2. Association and effect of rs7594645-G (*FASTKD2*) on immediate recall in the HRS discovery GWAS**

a) All SNPs within 500 kb of *FASTKD2* are plotted based on their GWAS  $-\log_{10} p$ -values, NCBI build 37 genomic position, and recombination rates calculated from the 1000 Genomes Project reference data. The color scale of  $r^2$  values is used to label SNPs based on their degree of LD with rs7594645. Genes in the region are labeled with arrows denoting 5'-to-3' orientation. b) Mean immediate recall scores (adjusted for clinical covariates and population structure)  $\pm$  standard errors are displayed based on rs7594645 genotype. Presence of the minor allele (G) of rs7594645 imparted a modest additive effect of improving memory performance and accounted for 0.5% of the phenotypic variance.



**Figure 3. Effect of *FASTKD2* rs7594645-G on hippocampal volume and gray matter density in 315 older healthy control participants from the ADNI**

Using high resolution T1-weighted structural MRI, mean volumes and gray matter densities in the hippocampus (adjusted for age, gender, education, and intracranial volume)  $\pm$  standard errors are displayed based on rs7594645 genotype. Participants with the minor allele (G) of rs7594645 displayed increased hippocampal volume and gray matter density, with a significant multivariate effect of genotype on these MRI measures ( $p=0.036$ ).

**Table 1**

Selected characteristics (number or mean (standard deviation)) of participants in the discovery\* and replication cohorts.

	HRS* (wave 3)	HRS (wave 7)	Add-Neuro-Med	ADNI	IMAS	MAP	ROS
<b>Participants</b>	6,705	5,116	64	1,147	68	878	803
<b>Gender</b>							
<b>Male</b>	2,529 (38%)	2,177 (43%)	27 (42%)	669 (58%)	25 (37%)	244 (28%)	276 (34%)
<b>Female</b>	4,176 (62%)	2,939 (57%)	37 (58%)	478 (42%)	43 (63%)	634 (72%)	527 (66%)
<b>Age (y)</b>	61.8 (8.2)	60.4 (10.5)	70.9 (6.8)	74.3 (7.1)	71.9 (8.1)	81.0 (6.6)	75.7 (7.3)
<b>Education (y)</b>	12.4 (3.1)	13.0 (3.1)	10.2 (5.0)	15.8 (2.9)	16.5 (2.5)	14.7 (2.9)	18.2 (3.4)
<b>Ethnicity</b>							
<b>NHC</b>	5,283	4,042	64	1,147	68	878	803
<b>AA</b>	817	711	0	0	0	0	0
<b>Other</b>	605	363	0	0	0	0	0

Abbreviations: NHC = non-Hispanic Caucasian; AA = African-American

**Table 2**

Biological pathways associated with immediate recall in the HRS discovery GWAS ( $n=6,705$ ).

Pathway Description (Source Database)	Size *	Uncorrected $p$ **
Valine, leucine, and isoleucine biosynthesis (KEGG)	11	$3.78 \times 10^{-9}$
IFN $\gamma$ pathway (Protein Interaction Database)	40	$1.21 \times 10^{-6}$
GA12 pathway (Signal Transduction Knowledge Environment)	23	$3.24 \times 10^{-6}$
MTORC1-mediated signaling (Reactome)	11	$3.28 \times 10^{-6}$
CDC42 pathway (Protein Interaction Database)	70	$5.48 \times 10^{-6}$
Mitochondrial tRNA aminoacylation (Reactome)	21	$1.45 \times 10^{-5}$
B-cell survival pathway (Biocarta)	16	$2.27 \times 10^{-5}$
CD28 co-stimulation pathway (Reactome)	32	$2.33 \times 10^{-5}$
CTCF pathway (Biocarta)	23	$4.12 \times 10^{-5}$
ErbB2/ErbB3 pathway (Protein Interaction Database)	44	$4.27 \times 10^{-5}$
Highly calcium-permeable postsynaptic nicotinic acetylcholine receptors (Reactome)	13	$6.05 \times 10^{-5}$
ErbB1 downstream pathway (Protein Interaction Database)	105	$8.48 \times 10^{-5}$
Creation of C4 and C2 activators (Reactome)	10	$1.16 \times 10^{-4}$
G1 phase (Reactome)	38	$1.48 \times 10^{-4}$
L1CAM interactions (Reactome)	86	$2.08 \times 10^{-4}$
Glioma (KEGG)	65	$2.48 \times 10^{-4}$
Acetylcholine binding and downstream events (Reactome)	16	$4.80 \times 10^{-4}$
Protein kinase B mediated events (Reactome)	29	$5.04 \times 10^{-4}$
Endocytosis (KEGG)	183	$5.85 \times 10^{-4}$
LKB1 pathway (Protein Interaction Database)	47	$6.27 \times 10^{-4}$
Nectin pathway (Protein Interaction Database)	30	$6.83 \times 10^{-4}$
Prion diseases (KEGG)	35	$6.83 \times 10^{-4}$

\* Number of genes in the pathway

\*\* All pathways displayed are significant at FDR-corrected  $p < 0.05$